

# Fern Laminar Scales Protect Against Photoinhibition from Excess Light

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**ABSTRACT.**—The laminar scales of the fern *Elaphoglossum paleaceum* were studied to determine if they act as a morphological mechanism to protect leaves from excess light. We hypothesized that if scales serve this purpose, then individual plants growing in high light would have greater laminar scale density than those in low light. For our first experiment, plants from high light roadsides were collected and subjected to artificial scale removal and then exposed to super-saturating pulse of light for 30 min. We found that leaves with their scales removed exhibited significantly greater photoinhibition than leaves with scales intact. Leaves with intact scales recovered fully after twelve hours whereas those with scales removed remained photoinhibited. Scale density on the adaxial side of leaves was positively correlated with light intensity. The data from this study indicate that fern laminar scales help reduce photoinhibition and potentially function as a morphological defense against photodamage.

The function of leaf vestiture has long been a botanical curiosity. Apart from their utility for taxonomic purposes, leaf hairs and scales play many roles that directly influence plant physiology (Karabourniotis, Kyparissis, and Manetas, 1993; Lambers, Chapin III, and Pons, 1998; Karabourniotis and Bornman, 1999; Manetas, 2003; Liakopoulos, Stavrianakou, and Karabourniotis, 2006). One commonly cited mechanism is the modification of leaf boundary layers (Lambers, Chapin III, and Pons, 1998). However, leaf vestiture can also directly influence physiology related to light and can alter the internal light environment of a leaf (Karabourniotis and Bornman, 1999), and reflect visible, infra-red, or harmful UV radiation (Manetas, 2003). There may also be important tradeoffs in that while photosynthesis is reduced in pubescent leaves relative to glabrous leaves, pubescent leaves are often protected from

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overheating, excessive water loss (Ehleringer and Bjorkman, 1978) and the potentially harmful effects of excessive light (Ripley, Pammenter, and Smith, 1999; Morales et al., 2002; Manetas, 2003).

Exposure to excessive light can have negative effects at multiple physiological levels. When a leaf is exposed to light, this energy can be transferred to three pathways: photosynthesis, heat, and fluorescence. While directing energy into photosynthesis is the most beneficial pathway for plants, we will focus on fluorescence in this paper. Plant physiologists use measures of fluorescence as an indicator of chlorophyll stress specifically in relation to photosystem II. Greater fluorescence results when photosystem II (PSII) reaction centers are closed or damaged and can no longer accept additional electrons, thus interrupting electron transport. Reaction centers close when they receive light in excess of that which they can transfer. Plants can dispose of this excess light energy various biochemical mechanisms. If the mechanisms in place to deal with excessive light are inadequate, photosystems can become damaged (extreme damage results in the commonly observed bleaching effect). Both excess light alone and photodamage result in Photoinhibition: the decline in photosynthesis due to exposure to excess light (Powles, 1984; Demmig *et al.*, 1987; Krause, Virgo, and Winter, 1995; Lambers, Chapin III, and Pons, 1998). If damage to the photosynthetic machinery has not occurred, photoinhibition is easily reversed following short recovery times (hours). However, if photodamage has occurred, photosynthetic recovery may take days (Demmig-Adams and Adams, 1996).

The ability of species to recover from the effects of stress associated with high light has been relatively well studied in tropical angiosperms (Mulkey and Pearcy, 1992; Lovelock, Jebb, and Osmond, 1994; Lovelock et al., 1998). In general, species recovery is closely correlated with that species' natural distribution. Lovelock *et al.* (1994) found that gap-dependent species recovered from high light stress faster than species found in dark understory habitats. A number of additional studies have demonstrated how excess light, especially when combined with other stresses, directly acts to limit species distributions (Chazdon, 1986; Groom, Baker, and Long, 1991; Kappen *et al.*, 1998; Kursar and Coley, 1999).

Given the ability of light stress to depress photosynthesis and to shape species distributions, it is not surprising that species have evolved a myriad of complex morphological and biochemical mechanisms that allow them to cope with light stress (Demmig-Adams and Adams III, 1992). These include morphological mechanisms such as leaf hairs (Karabourniotis, Bornman, and Liakoura, 1999), leaf movement or curling to avoid direct solar radiation when leaves are dry (Lebkuecher and Eickmeier, 1993; Sherwin and Farrant, 1998; Brighigna *et al.*, 2002) and protective biochemical mechanisms such as those that divert energy into pigment conversion (e.g., carotenoids and the xanthophyll cycle) (Demmig-Adams and Adams, 1996; Tausz, Hietz, and Briones, 2001a) or glycolate metabolism (Larcher, 2003).

Little is known about how ferns cope with excess light or what mechanisms they possess to do so. Biochemical mechanisms, such as xanthophyll-mediated



energy dissipation, have been tentatively demonstrated in the pteridophytes (Eickmeier, Casper, and Osmond, 1993; Tausz, Hietz, and Briones, 2001). One more commonly observed behavior in ferns is leaf curling. Leaf curling occurs in some pteridophytes as a direct result of leaf drying. Such leaf curling reduces the exposed evaporative surface which results in reduced drying rates. Yet, dehydrated leaves and excessive light can be a deadly combination. The added, and perhaps critical, benefit of leaf curling is the reduction of exposed photosynthetic tissue to incoming solar radiation. *Pleopeltis polypodioides* *P. vulgare*, and *Selaginella lepidophylla* have long been known to exhibit leaf curling (Lebkuecher and Eickmeier, 1991; Muslin and Homann, 1992; Lebkuecher and Eickmeier, 1993). In the case of *S. lepidophylla*, leaf curling has been shown to specifically limit both heat and light stress (Lebkuecher and Eickmeier, 1991, 1993). The ability of *Selaginella lepidophylla* to limit stress from light and drought has been shown to play a role in this species' ability to grow in desert like habitats (Eickmeier, Casper, and Osmond, 1993). Durand and Goldstein (2001) have also shown that native tree ferns in Hawaii exhibit greater photoinhibition when subjected to high light relative to an invasive and more widespread tree fern species. Both of these studies indicate that stress may play an important role in shaping species distributions.

One potential photoprotective mechanism that has not been examined thoroughly in the ferns is the presence of laminar scales. Laminar scales may reduce excess light thereby decreasing photoinhibition and limiting damage to photosynthetic machinery. We propose two hypotheses: 1) laminar scales act as a morphological photoprotective mechanism in the fern *Elaphoglossum paleaceum*, 2) scale density in this species will increase with increasing natural light. To test these hypotheses, we examined the effects on photoinhibition of experimental scale removal coupled with exposure of de-scaled leaves to excess light. We then examined the relationship of a naturally occurring and increasing light gradient in laminar scale density.

#### MATERIALS AND METHODS

This study was conducted at the Cuericí Biological Field Station in the San José Province, Costa Rica, *ca* 5 km northeast of the Pan American Highway (9°33'30"N, 83°39'42"W). The elevation is *ca* 2600 m, and the vegetation is upper montane wet forest (Holdridge, 1967). At this station, we studied the fern *Elaphoglossum paleaceum* (Elaphoglossaceae) (Fig. 1a) which is abundant along high-light roadsides and in the darker understory around the station. Taxonomically, the species falls within subgenus *Lepidoglossum* whose members commonly produce scales on the abaxial and adaxial surfaces of the leaf (Fig. 1b&c). These scales have no vascular connection to the sporophyte and are easily removed with no apparent damage to the sporophyte.

In the field, a 100 m transect was set up along a naturally occurring light gradient that began at a fully exposed roadside site and continued through an open area and into the forest. Three similarly aged sporophytes were collected



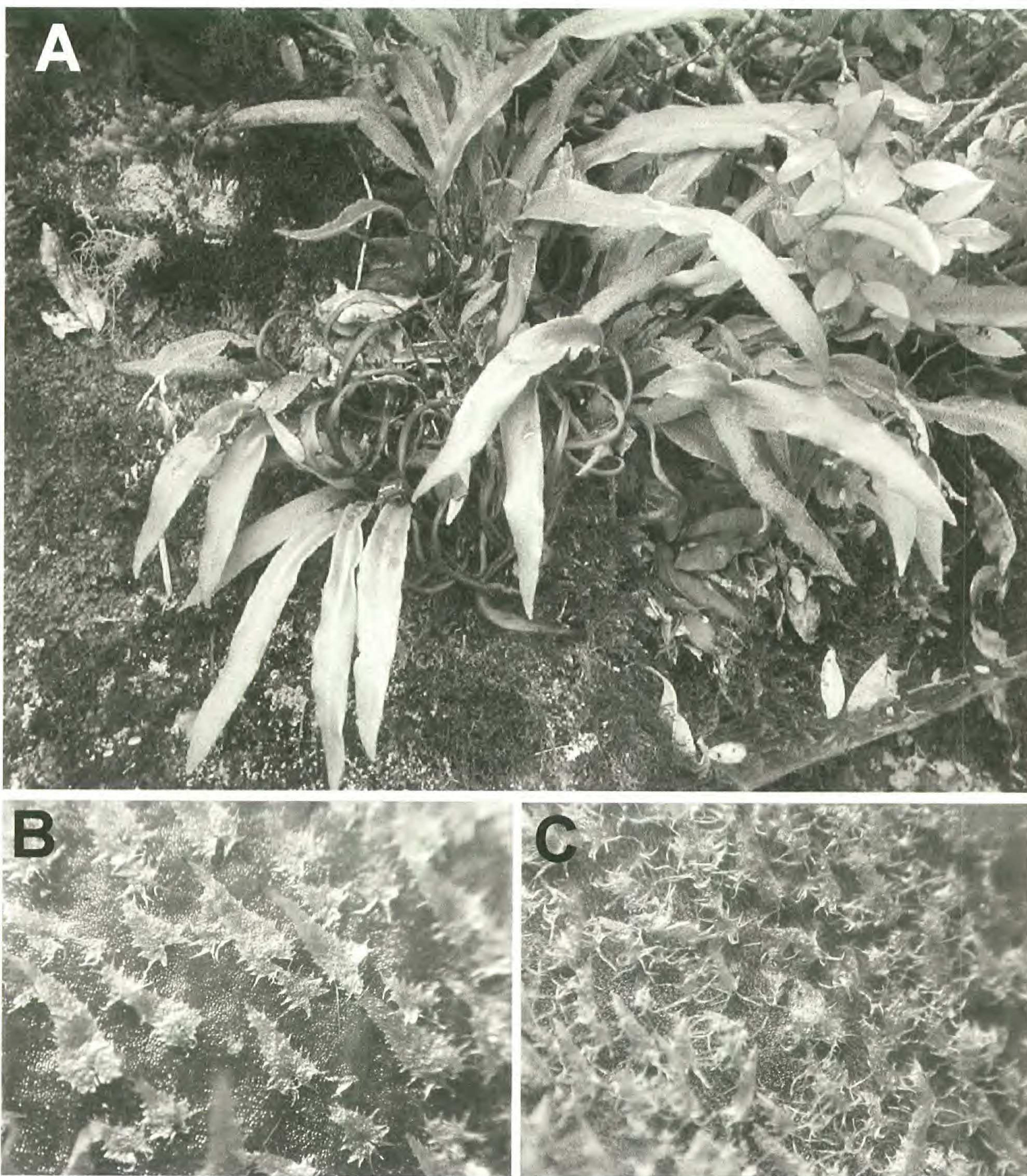


FIG. 1. *Elaphoglossum paleaceum*. A. Habit; this individual was photographed growing on a roadside in full sunlight around Cuericí Biological Field Station in Costa Rica. B. Scale morphology and density on the adaxial surface and of a typical sun leaf. C) Abaxial surface. B and C photographed at the same magnification.

from 20 of the nearest individuals at every 5 m interval along the 100 m light gradient. Scale density was quantified under a stereoscope on both the abaxial and adaxial surfaces of a 10 mm  $\times$  10 mm area from each of three sporophytes. An ANOVA was run to determine if there were differences in scale density among the leaves of individual plants. No differences were found and we therefore calculated the average abaxial and adaxial densities of each three leaf set.



To assess light environments, a digital hemispherical photograph was taken above each sampled individual with a Nikon Coolpix 950 digital camera (Melville, New York, USA) with a fisheye lens attachment. Photographs were then analyzed using the computer program Gap Light Analyzer (Frazer et al., 1999) to estimate the percentage of total transmittance. Regression analysis of the relationships between percent transmittance and scale density on both leaf surfaces was performed using the statistical software package JMP (SAS-Institute, 2005).

In order to determine whether scales act as a morphological photoprotective mechanism, ten plants were field collected from high light roadside environments with roots intact and placed in plastic bags containing 250 ml of water for two hours. Two leaves of similar ages were marked on each plant. On one frond, scales were removed using the blunt end of a spatula whereas scales on the other frond were left intact. To establish a baseline of leaf function on the marked fronds, we used an Opti-Sciences modulated fluorometer (OS-500) to measure chlorophyll fluorescence. For this measurement, leaves were placed in the dark for 20 min after which we took an initial dark-adapted measurement of chlorophyll fluorescence ( $F_v/F_m$ ). Plants were then exposed to a 30 min pulse of light at  $2000 \mu\text{mol}/\text{m}^2/\text{sec}^{-1}$ . The pulse of light was produced by MR16 Superline 50 w halogen bulbs with dichroic coated reflectors. Dichroic coated reflectors are made up of dozens of layers of thin materials that reduce the heat generated from the bulb by reflecting infrared light back into the reflector and away from the specimen.

Immediately following this exposure, chlorophyll fluorescence ( $F_v/F_m$ ) was again measured in order to determine the degree of inhibition relative the pre-treatment dark-adapted measurement of  $F_v/F_m$ . To determine the degree of recovery following exposure to the light treatment, plants were placed in the dark and another dark adapted ( $F_v/F_m$ ) measurement was taken 12 hr post light treatment. As a control, an additional set of plants had their scales removed but did not experience the light stress treatment. A two sample t-test was performed to determine if there were differences between the initial dark-adapted, post light stress (inhibition) treatment, and the 12 hr post treatment  $F_v/F_m$  measurements using JMP software (SAS-Institute, 2005).

Measurements of chlorophyll fluorescence have been widely used in studies to measure the general physiological condition or degree of stress that a plant may be experiencing. Standard chlorophyll fluorescence methods were followed in this study. The initial dark-adapted values of  $F_v/F_m$  mentioned above provide an estimate of the maximal quantum efficiency of Photosystem II, which in healthy, unstressed material universally converges around 0.76–0.83 (Bjorkman and Demmig, 1987). The degree of photoinhibition from the light treatment was determined by comparison to the initial dark-adapted value. Although photosynthesis is not measured directly, there is significant correlation across many studies to indicate that lowered  $F_v/F_m$  values result in lowered photosynthetic rates. Thus, measurements of  $F_v/F_m$  serve as a proxy for photosynthesis (Bjorkman and Demmig, 1987; Lambers, Chapin III, and Pons, 1998).



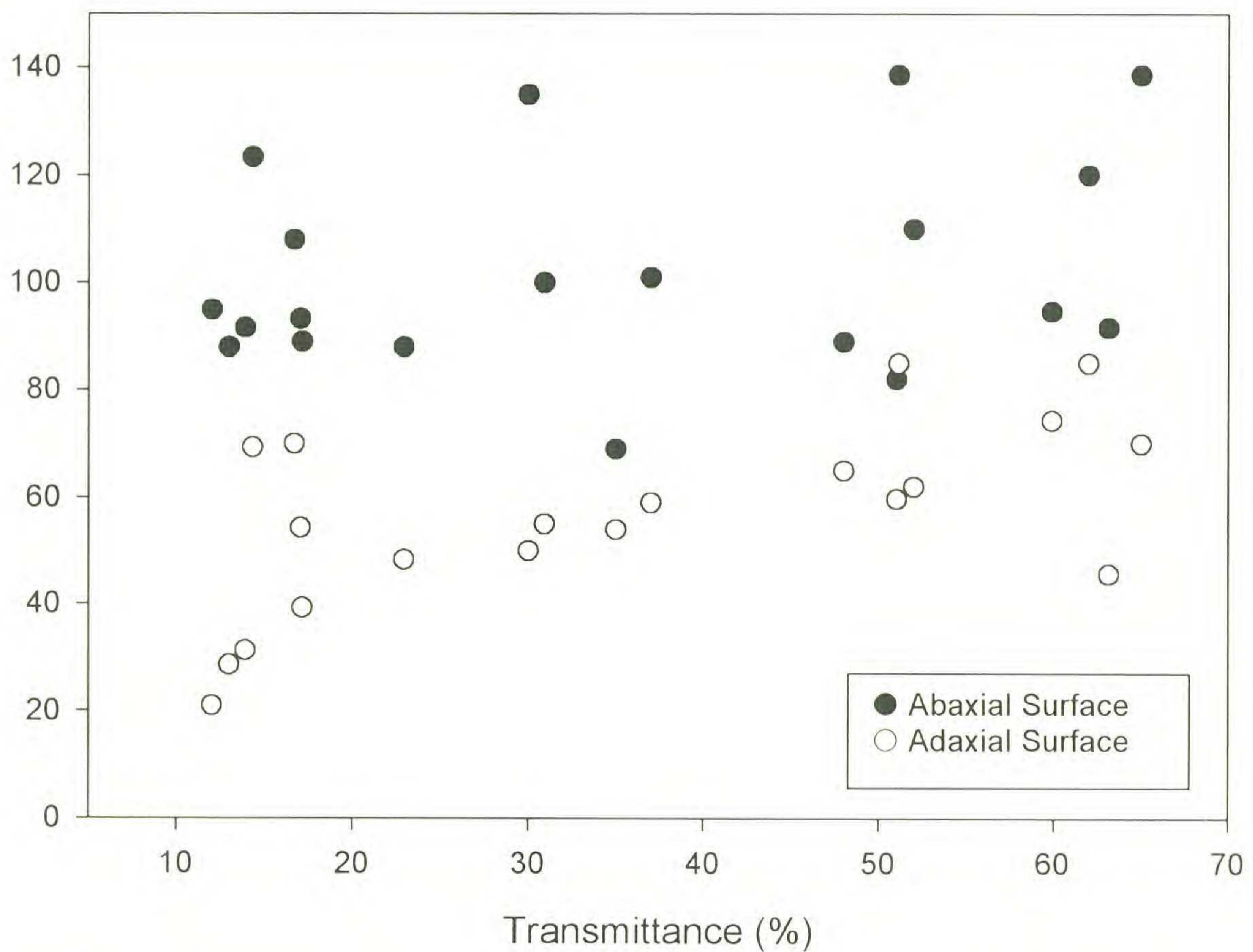


FIG. 2. The relationship between natural light transmittance and leaf scale density of *Elaphoglossum paleaceum*.

## RESULTS

Regardless of light intensity of the habitat, scale density was significantly higher on the abaxial (bottom surfaces, away from exposure) than on the adaxial (top surface, facing exposure) of the leaves (data not shown,  $t = 6.631$ ,  $p < 0.0001$ ). As predicted, we found that adaxial scale density increased with increasing percent transmittance along our sampled light gradient (Fig. 2). The relationship was significant with a linear regression ( $r^2 = 0.3843$ ,  $p = 0.0035$ ), but exhibited a slightly better fit with a second order polynomial regression ( $r^2 = 0.409$ ,  $p = 0.0115$ ). There appeared to be no clear relationship between abaxial scale density and percent light (Fig. 2; linear regression,  $r^2 = 0.0689$ ,  $p = 0.2633$ ).

Initial dark adapted measurements indicated that the leaves were healthy and not experiencing stress due to transplantation into plastic bags (Fig 3a,  $n = 10$ ,  $p = 0.421$ ). After exposure to supersaturating light, the leaves with scales intact exhibited significantly less photoinhibition than plants with the scales removed (Fig. 3b,  $n = 10$ ,  $p < 0.0001$ ). Following the 12 hour post stress recovery period, leaves with scales intact exhibited significantly greater recovery than leaves with the scales removed (Fig. 3c,  $n = 10$ ,  $p < 0.0001$ ). There was also no significant photoinhibition in control plants with scales removed but not exposed to light stress ( $n = 10$ ,  $p = 0.524$ ).



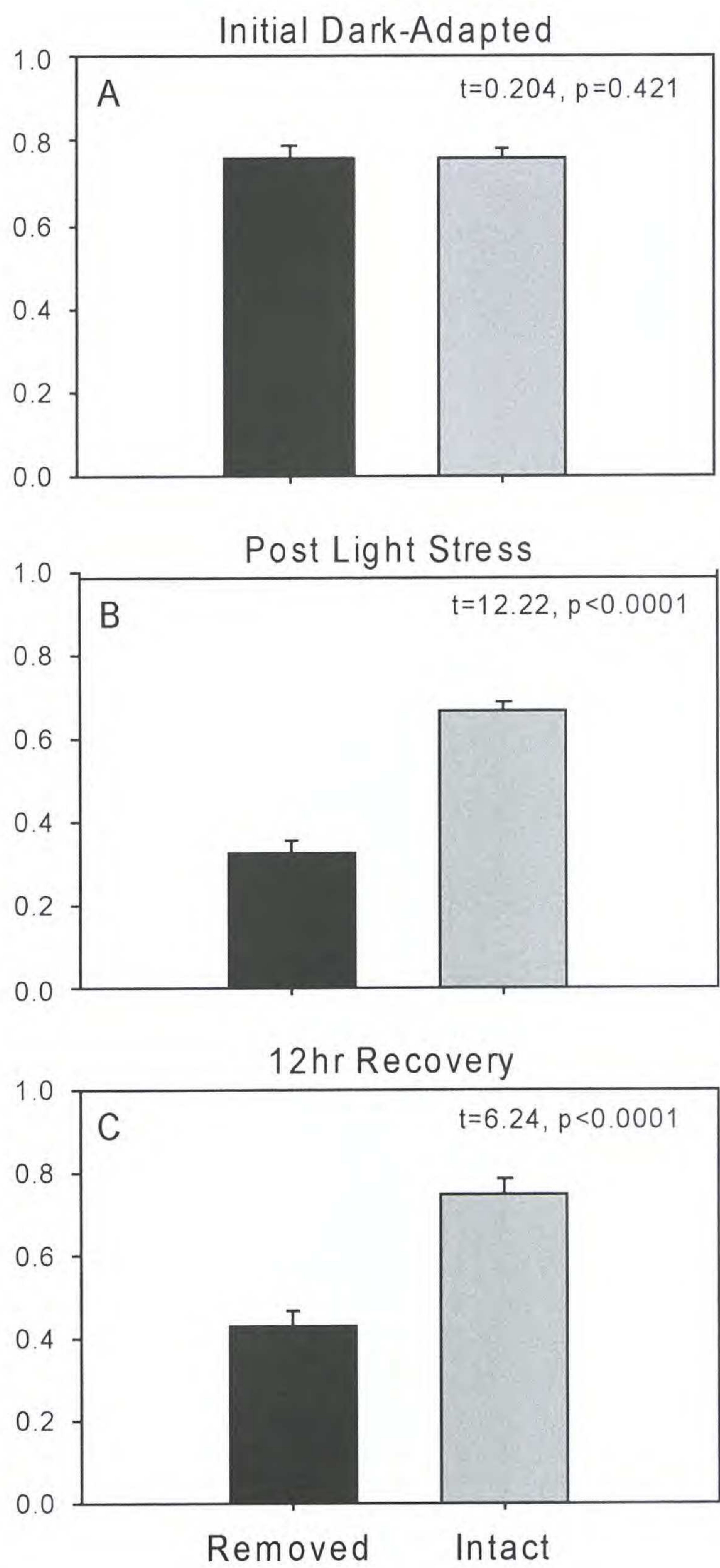


FIG. 3. Changes in photosystem II yield ( $F_v/F_m$ ) from fronds of *Elaphoglossum paleaceum* with scales intact and with scales experimentally removed. A. Initial 20 min. dark adapted control values. B.  $F_v/F_m$  values after 30 min. exposure to a light level of  $2000 \text{ } \mu\text{mol}/\text{m}^{-2}/\text{sec}^{-1}$ . C. Recovery values of  $F_v/F_m$  12 hours post light stress. A two sample t-test ( $n = 10$ ) was performed to compute differences between leaves with and without scales. Error bars are standard errors.



## DISCUSSION

The data from our study suggest a novel role for fern laminar scales: that of photoprotection. Plants where the scales were removed had significantly lower  $F_v/F_m$  values after high light stress, which is indicative of greater photoinhibition, compared to leaves with scales intact. Additionally, leaves with scales intact exhibited slightly, but not significantly lower  $F_v/F_m$  values after high light stress in comparison to the dark-adapted values (Fig. 3a intact compared to Fig. 3b intact values; analysis not shown). The significantly reduced rate of  $F_v/F_m$  recovery in leaves where scales have been removed (Fig. 1c) indicates that these plants likely experienced photodamage. Taken together, these data indicate that the presence of fern laminar scales in *Elaphoglossum paleaceum* clearly protects plants from photoinhibition and photodamage that occur when plants are exposed to excess light.

This hypothesis is further supported by the positive relationship of scale density with natural light intensity. Adaxial scale density was positively correlated with an individual plant's natural light regime where plants in high light had greater scale density than plants in low light. This follows our prediction that if laminar scales act as a morphological photoprotective mechanism then plants in high light habitats should exhibit greater investment in such structures.

*Elaphoglossum paleaceum* is additionally intriguing in that it exhibits leaf curling during times of extreme drought (pers. obs.). The differential pattern of scale density between the abaxial and adaxial surfaces combined with leaf curling may be explained as a last line of morphological defense in times of drought. During leaf curling, the abaxial surface curls upwards forming a tube that completely hides the adaxial surface (pers. obs.). This mechanism presents the more densely covered abaxial surface to full exposure to incoming solar radiation. This curling can reduce surface exposure and slow evapotranspiration and thus potentially reduce or eliminate the damaging effects of extreme light. In times of high light without drought or with limited drought, adaxial scale density may be sufficient to protect the plant from extreme photoinhibition and photodamage. The interaction of scale density with light intensity suggests that variation in this character may be driven by light and provides some adaptive value.

There are numerous other species of *Elaphoglossum* in the flora of this region that lack scales and leaf curling, yet grow alongside *Elaphoglossum paleaceum*. It is likely that different strategies have evolved among these species with the glabrous taxa relying on biochemical mechanisms. Future studies would benefit greatly from inclusion of these species and from investigation of protective pigments.

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